Visual and fluorogenic detection of a nerve agent simulant *via* a Lossen rearrangement of rhodamine–hydroxamate†

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A visual and fluorogenic detection method for a nerve agent simulant was developed based on a Lossen rearrangement of rhodamine–hydroxamate, in the presence of diethyl chlorophosphate, under alkaline conditions.

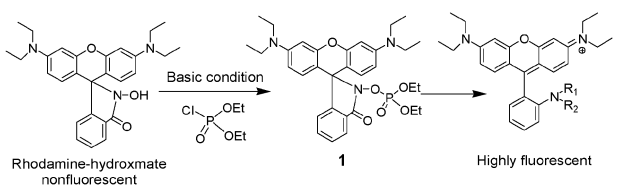
Nerve agents, including Tabun, Sarin, and Soman, are chemically active organophosphates that can inactivate acetylcholinesterase *via* irreversible modification of the catalytically essential serine residue in the enzyme active site.[1] Inhibition of acetylcholinesterase leads to accumulation of acetylcholine, which leads to neuromuscular paralysis and eventually death.[2,3] The utility of nerve agents stems from their extraordinary toxicity; a lethal dose can be as little as 0.70 mg for an average 70 kg man. Nerve agents are an increasing homeland security concern due to the horrifying effects that could be realized from a terrorist attack. Sarin is probably the most feared chemical agent because it has actually been used by terrorists. In 1995, the Aum Shinrikyo group released Sarin gas in the Tokyo subway, leading to thousands of wounded and 12 deaths.[4] As such, methods allowing facile and sensitive detection of nerve agents will be desirable to both government officials and the general public.

Systems that have been utilized to detect nerve agents include liquid chromatography/mass spectrometry, enzyme based biosensors *etc*.[5–7] These conventional methods usually show limitations, such as lack of portability or storage/stability issues that limits their success in some conditions. The challenge to the scientific community is to invent methods and devices that are compact, portable and capable of real time detection. Chemosensors are advantageous in the aforementioned aspects as they require widely used instruments and offer the possibility to detect nerve agents with the naked eye. Chemosensors that exhibit either distinguished fluorescence emission properties or change color in the presence of nerve agents have been extensively investigated.[8–21] In some chromogenic/fluoregenic assays, carefully designed substrates could react with nerve agent mimics to directly afford either detectable species,[19,21] or to form intermediates, which could further undergo intramolecular transformations *in situ* to generate chromophores or fluorophores for signal detection.[10,19–21] Alternatively, in other assay systems,[11,12,14,17,20] selected fluorophores were covalently attached or complexed with fluorescence quenchers. Upon reaction with nerve agents, the quenching effects were diminished or inhibited, allowing the recovery of the masked fluorescence.

Selected functional groups, mainly amino,[11] hydroxyl,[10,12,17,20] or oxime moieties,[13,16,19,21,22] have been employed as the initial anchoring points for nerve agent simulants in many of the reported chemical reagent based assays.

Herein, we report a new nerve agent sensor, which contains a hydroxamate group that acts as a nucleophile and reacts with organophosphate. Rhodamine B was treated with hydroxylamine to give nonfluorescent rhodamine–hydroxymate under basic conditions. The hallmark of rhodamine–hydroxamate is the formation of the intramolecular 5-membered spirolactam (Scheme 1). Upon addition of diethyl chlorophosphate which was used as the nerve agent simulant, the rhodamine–hydroxymate solution gradually turned red in color (Fig. 1). Historically, nonfluorescent and colorless rhodamine derivatives with spirolactam have been extensively utilized to detect metal ions by virtue of reversible ring-opening of the spirolactam, which gives rise to a highly fluorescent and colored rhodamine fluorophore.[23–27] Particularly, the hydroxamic acid derivatives of rhodamine B and rhodamine 6G have been evaluated for fluorescent imaging of cellular hypochlorous acid by oxidation promoted irreversible ring opening of the spirolactam.[28] Herein, we report the irreversible ring opening of rhodamine spirolactam *via* a Lossen rearrangement in the presence of reactive organophosphate.

To optimize the assay, the reaction rates of diethyl chlorophosphate with rhodamine–hydroxamate under a variety of conditions were monitored with UV-vis absorbance at 590 nm. Time course studies of the reaction rates showed that dimethylformaldehyde (DMF) was the preferred assay medium relative to other less polar solvents, and the assay was catalyzed efficiently in DMF containing a variety of bases such as triethylamine (TEA), piperidine *etc*. Under the examined conditions, color development was found to be proportional to the incubation time, indicating that the opening of the spirolactam of compound **1** was the rate limiting step in the assay conditions. The assay was as efficient in the presence of up to 5% (v/v) water included in the reaction solution as that in anhydrous DMF (ESI, SFig. 4†). The tolerance of water in



Scheme 1 Fluorogenic and chromogenic reaction of rhodamine–hydroxamate with diethyl chlorophosphate.

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† Electronic supplementary information (ESI) available: Synthesis and characterization of rhodamine–hydroxamate, fluorescence spectra of the colored species and assay procedure for diethyl cholorophosphate. See DOI: 10.1039/c0cc02881a

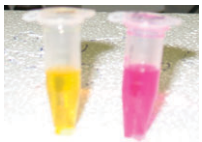


Fig. 1 Comparison of rhodamine–hydroxamate solution (1 mg mL^{-1}) in DMF containing TEA (3%, v/v) before (left) and after (right) addition of diethyl chlorophosphate (250 ppm).

the assay system is probably due to quicker formation of compound **1** relative to the hydrolysis of diethyl chlorophosphate. The effectiveness of the assay in the presence of water suggests its robustness in monitoring nerve agents under selected practical conditions.

To test the sensitivity of the rhodamine–hydroxamate based assay towards nerve agents, diethyl chlorophosphate was added to the solution of rhodamine–hydroxamate (1 mg mL^{-1}) in DMF containing TEA (3%, v/v) to prepare a serial of reaction solutions with various concentrations of the analyst. The reactions were incubated at room temperature for 20 min and then the fluorescence emission spectra recorded (Ex: 560 nm). As can been seen from Fig. 2, the maximum fluorescence emission intensity at 590 nm increased as a function of diethyl chlorophosphate concentration. An amount of diethyl chlorophosphate as low as 25 ppm can be clearly identified under the assay condition (510 RFU relative to the background of 90 RFU). Since the color of the reaction solution was developed in a time dependant manner, the assay limits could be further extended by elongation of the incubation time.

The reaction of rhodamine–hydroxamate with diethyl chlorophosphate was monitored with thin layer chromatography. It was shown that rhodamine–hydroxamate was consumed in minutes upon addition of slightly excess diethyl chlorophosphate. A colorless intermediate adduct was quickly formed, while several colored species appeared and gradually accumulated. To access the identity of the intermediate adduct, which is presumably compound **1** (Scheme 1), the reaction solution was analyzed by high resolution mass spectrometry (HRMS), Fig. 3. A major peak located at 594.2739 was identified, which is consistent with the theoretical molecular weight of compound **1** ($C_{32}H_{41}N_3O_6P+$; MW: 594.2733), confirming formation of compound **1** in the assay system. The UV-vis

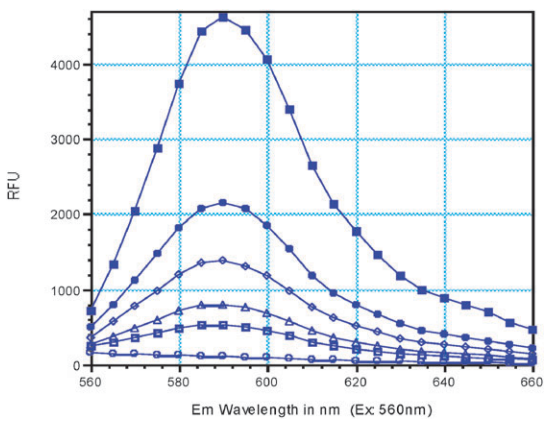


Fig. 2 Fluorescence emission spectra of diethyl chlorophosphate detected with rhodamine–hydroxamate. Analyst concentration utilized: 500, 170, 100, 50, 25, and 0 ppm (From top to the bottom).

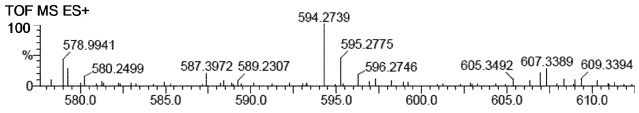
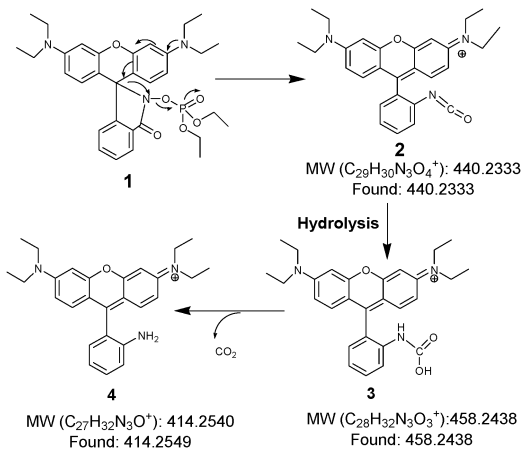


Fig. 3 HRMS confirmation of compound **1** in rhodamine–hydroxamate based assay solution of diethyl chlorophosphate.

absorption spectrum of the reaction solution revealed a new strong absorption band at 560 nm, which intensified as a function of time (ESI, SFig. 3 and 4†). The fluorescence excitation and emission spectra of the resulting solution were shown to be almost identical to that of rhodamine B (ESI, SFig. 1 and 2†), indicating the formation of a rhodamine fluorophore from the ring opening of spirolactam in compound **1** under the assay conditions (Scheme 2).

Known as the Lossen rearrangement, hydroxamic acids are able to be converted to O-acyl, sulfonyl, or phosphoryl intermediates, which will then self-rearrange into the corresponding isocyanates.[29–33] We reasoned that ring opening of the spirolactam in compound **1** might be performed *via* a Lossen rearrangement. Based on this hypothesis, compound **2** with an isocyanate functionality will be formed from compound **1** (Scheme 2). As the isocyanate group is chemically very reactive, it is prone to further reactions with a variety of nucleophiles. In the presence of water, compound **2** will be hydrolyzed to afford compound **3**, which will decompose to liberate a molecule of carbon dioxide to yield compound **4**. Formation of compound **2**, **3** and **4**, which share a common structural motif of the fluorescent and colored rhodamine fluorophore, would contribute to the formation of the observed colored species in the assay. To probe the molecular identities of the colored species generated in the rhodamine–hydroxamate based assay solution, the reaction solution was subjected to high resolution mass spectrometry analysis. As shown in Scheme 2 and Fig. 4, peaks representative of compound **2**, **3** and **4** were all shown to be present in the reaction solution, supporting the hypothesis that the color development was achieved through a Lossen rearrangement of compound **1**. Besides O-phosphoryl derivatives of hydroxamate, the O-acyl, and sulfonyl intermediates of hydroxamate are also poised to undergo Lossen rearrangement.[29–33] In a separate experiment,



Scheme 2 Proposed intermediates generated *via* a Lossen rearrangement in rhodamine–hydroxamate based assay solution for diethyl chlorophosphate.

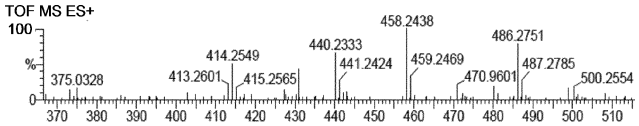


Fig. 4 HRMS analysis of possible intermediates generated from diethyl chlorophosphate initiated a Lossen rearrangement of rhodamine–hydroxamate.

color developments of rhodamine–hydroxamate in the presence of 4-toluenesulfonyl chloride or benzyl chloroformate were also observed, supporting the role of the Lossen rearrangement in the assay. The highly fluorescent and deep colored properties of the intermediates resulting from the Lossen rearrangement allow instrumental quantitation as well as 'naked-eye' detection of a broad range of reactive organophosphates.

It is preferential to detect ppb level of nerve agents in the gas phase for practical applications. Here we show that rhodamine–hydroxamate is able to assay a single aliquot of diethyl chlorophosphate in DMF solution at ppm range. In practical applications, detection of a lower level of nerve agent might be achieved by bubbling a larger volume of contaminated air into the assay solution. Currently the conjugation of rhodamine–hydroxmate with functional solid materials are being investigated for detection of gas phase nerve agents with enhanced sensitivity. Organophosphate promoted Lossen rearrangement of rhodamine–hydroxmate offers a fluorogenic and colorimetric chemical transformation that might be useful for design of a nerve agent assay kit.

In summary, a visual and highly fluorescent detection method for a nerve agent simulant was developed based on a Lossen rearrangement of rhodamine–hydroxamate in the presence of reactive organophosphate. The assay was sensitive enough to detect 25 ppm of nerve agent mimics in 20 min, and was robust enough to be assayed under humid conditions, suggesting its potential utility for on-spot detection of nerve agents with the aid of instruments or possibly with the 'naked eye'.

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